Effects of Fatty Acids, Fatty Amines and Propylene Glycol on Rat Stratum Corneum Lipids and Proteins *in Vitro* Measured by Fourier Transform Infrared/Attenuated Total Reflection (FT-IR/ATR) Spectroscopy

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Fourier transform infrared/attenuated total reflection (FT-IR/ATR) spectroscopy was used to examine the effect of fatty acids, fatty amines and propylene glycol (PG) on the molecular mobility of rat stratum corneum lipids and keratinized proteins, using a hydrophobic solute, indomethacin, and a polar solute, 5- and 6-carboxyfluorescein (CF). Treatment of the skin with either oleic acid or oleylamine resulted in significant CH_2 C–H asymmetric stretching band shifts and broadening. The extent of spectral alteration varied with the chemical structure of the penetrant. The penetrants increased the lipophilic indomethacin flux and shortened the lag times through the skin *in vitro*. The plot of frequency changes *vs.* indomethacin flux or lag time demonstrated a linear relationship, thus indicating that spectral alteration in CH_2 C–H stretching regions of stratum corneum lipids may provide a reliable index for characterizing penetrants. The data also showed that the hydrophilic group which attached to the CH_2 group in the penetrant molecules did not play a part in the membrane permeability enhancing action. Oleic acid and oleylamine appeared to induce a conformational alteration of the keratinized proteins from α-helix to beta sheet. Such alteration was also observed with PG treatment. Accumulation of CF was significantly increased by the PG pretreatment of the skin, thus suggesting that PG-induced protein conformational changes could be related to the enhancement of CF accumulation.

Keywords skin penetration enhancer; fatty acid; fatty amine; propylene glycol; stratum corneum; stratum corneum perturbation; FT-IR/ATR spectroscopy

Introduction

The stratum corneum consists of cornified cells embedded within a matrix of lipid bilayers.[1] The stratum corneum is a significant barrier to the percutaneous flux of most drugs.[2] Percutaneous enhancers have been used to increase drug flux by altering the stratum corneum barrier.[3]

Oleic acid has been hypothesized to enhance skin penetration by disordering the stratum corneum lipids.[4-6] Mak *et al.* studied the penetration of 4-cyanophenol (CP), a model permeant, and the effect of oleic acid on permeant flux through the stratum corneum, as well as oleic acid effects on the stratum corneum using *in vivo* Fourier transform infrared/attenuated total reflection (FT-IR/ATR). They concluded that oleic acid disordered the stratum corneum intercellular lipid domains, resulting in an enhanced penetration of CP.[5] Similar membrane alterations have been observed in the presence of other *cis*-unsaturated fatty acids[4] and *n*-alkanols.[7] Transmission FT-IR analysis of excised porcine stratum corneum demonstrated that octadecenoic acid (oleic acid) caused a significant shift of the CH_2 C–H asymmetric stretching band near 2920 cm^{-1} toward higher wavenumbers, suggesting that these penetrants incorporated within the stratum corneum disrupted lipid packing.[4] In this report, the author also stated that the percutaneous flux of salicylic acid was related to these changes in lipid packing. Such observations described the usefulness of assessing frequency changes in the CH_2 C–H asymmetric stretching band in evaluating the characteristics of transdermal enhancers.

In this study, we compare the effects of fatty acids and fatty amines on stratum corneum structures, and examine whether the FT-IR/ATR technique could quantitatively predict the ability of these penetrants to increase stratum corneum fluidization. For this purpose, frequency changes due primarily to the CH_2 C–H asymmetric stretching band near 2920 cm^{-1} in the stratum corneum lipids treated either with or without penetrants in the presence of propylene glycol (PG) were used as an index to evaluate the above mentioned characteristic changes. The molecular mobility of the keratinized proteins was also examined. Indomethacin flux was also determined to relate to the above frequency changes. Oleic acid has been commonly used as penetrant, therefore several fatty acids and their structural analog, fatty amines, were selected as penetrants to examine the effect of structural changes of these compounds on the disordering of stratum corneum lipid domains. PG used as the solvent for both the penetrants and indomethacin was also examined for its influence on the stratum corneum structures and the flux of hydrophilic solutes, 5- and 6-carboxyfluorescein, through the skin.

Materials and Methods

Materials Lauric, myristic, palmitic, stearic and oleic acids and lauryl, myristyl, hexadecyl, and oleyl amines were purchased from Nakarai Tesque. Stearylamine was purchased from Wako Junyaku Kogyo. All of these chemicals were of a reagent grade. Azone was kindly supplied from Nelson Research Center. Propylene glycol was obtained from Nakarai Tesque. 5- and 6-Carboxyfluorescein (CF) and indomethacin were obtained from Molecular Probes Inc. and Sumitomo Pharmaceuticals, respectively. All other chemicals were also of a reagent grade.

Skin Preparations for FT-IR/ATR Spectroscopy Measurement As previously reported,[8] the abdominal skin was removed from male Wistar rats (8—9 weeks old) under pentobarbital anesthesia, shaved with an electric clipper and then with an electric razor. The freshly excised full thickness skin with subcutaneous fat removed was weighed. The skin surface area available for FT-IR/ATR measurement was 1.05 cm^2. The skin samples were then incubated in PG with or without the penetrant for 2 h at 37 °C. After the incubation, the skin surface was gently wiped with Kimwipes® and then rinsed with ethanol for 10 s. This sample was then vacuum dried for 1 h. The control sample was prepared in an identical manner without PG. The 1% indomethacin–PG solution was also applied to the excised surface of the abdominal skin to examine the effect of indomethacin on stratum corneum structures. The samples were hydrated before use in a chamber maintained at 95% relative humidity at

25 °C for 3 d. Under these conditions, the skin samples equilibrated to a water content of about 50%. Each hydrated skin sample was weighed after the above treatment, and the water content in the skin, evaluated according to Eq. 1, was approximately 579.3 ± 3.5 mg/g.

$$\% \text{ water content} = \frac{\text{hydrated} - \text{decicated}}{\text{hydrated}} \times 100 \quad (1)$$

FT-IR/ATR Spectroscopy Measurement IR spectra of the stratum corneum were obtained at an ambient temperature with a JEOL JTR-100 FT-IR spectrometer equipped with a liquid nitrogen cooled, narrow band mercury–cadmium–telluride detector (MCT detector) with a resolution of 0.45 cm^{-1}. An internal reflection element was KRS-5 (52 × 20 × 2 mm trapezoid cut at 45°). The internal reflection element permitted the infrared beam to a depth of between 0.58 μm (4000 cm^{-1}) and 5.8 μm (400 cm^{-1}) into the stratum corneum, which is about 20 μm thick.[9] The depth of penetration was estimated according to Eq. 2.[10]

$$\frac{d_p}{\lambda} = \frac{1}{2}\pi n_1 \left[\sin^2\theta - \left(\frac{n_2}{n_1}\right)^2 \right]^{-1/2} \quad (2)$$

where d_p is the depth of penetration. The symbols, λ, θ, n_1 and n_2 were the wavenumbers to measure the spectra, an incident angle (45°), refractive index of KRS-5 (2.4) and the refractive index of the stratum corneum (1.55),[11] respectively. The CH_2 C–H asymmetric stretching band peak originated from alkyl chains in the lipids were obtained by the built-in programmed curve fitting method of the FT-IR/ATR instrument.

***In Vitro* Permeability** Indomethacin: A freshly-excised full thickness rat abdominal skin obtained under pentobarbital anesthesia was mounted between the two compartments of the diffusion cells with the dermis side facing the receiver compartment. The diffusional area between the two compartments was 1.05 cm^2. The formulations used were 1% w/w indomethacin in PG without any buffer solution either with or without 0.15 M fatty acid, fatty amine or Azone. One gram of the vehicle was applied into the donor compartment. The donor compartment was sealed from the atmosphere with Parafilm®. The reservoir compartment was filled with 14.2 ml of a phosphate buffered solution (PBS; 140 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4 and 1.47 mM KH_2PO_4, pH 7.4). The assembled diffusion cells were then immediately immersed in a water bath at 37 °C and stirred with a magnetic stirrer. The receiver compartments were maintained at 37 °C. Samples (0.3 ml) were taken at appropriate intervals from the receiver compartments and replaced with PBS maintained at 37 °C. The indomethacin concentrations were assayed by fluorometry as described by Hucker *et al.*[12] A Hitachi F-3000 Fluorometer was used to determine the concentrations of indomethacin across the skin. The permeation profiles were constructed by plotting the cumulative amount of indomethacin permeated *vs.* time, and the steady state rates of indomethacin (flux) and lag time were calculated.

5- and 6-Carboxyfluorescein: The hydrophilic drug, CF, was selected to determine a polar solute's accumulation in the full thickness skin. The skin was mounted between two compartments of the two chamber diffusion cell as before. The epidermal surface was pretreated with either PBS (pH 7.4) or PG for 2 h. After this treatment, the receiver solution was filled with fresh PBS (pH 7.4), and then 1 g of 0.02% CF in PBS (pH 7.4) was introduced into the donor compartment. After 2 h incubation, the skin was removed from the diffusion cell and the epidermis surface was gently cleansed as described earlier. The obtained skin samples (full thickness skin) were then homogenized (Physcotron, Nichion Irika Kikai Seisakusho NS-50). The homogenates were filtered and then the filtrates were assayed for CF according to the method reported by Ohsima *et al.*[13]

Results and Discussion

The FT-IR/ATR spectra of the surface of rat skins which have been treated with (a) 0.15 M oleylamine in PG, (b) 0.15 M oleic acid in PG, (c) 1% indomethacin in PG, and (d) PG alone, and of (e) untreated skin are illustrated in Fig. 1. The CH_2 C–H asymmetric and symmetric stretching vibrations absorbing near 2920 and 2860 cm^{-1}, respectively, result primarily from the methylene groups of the stratum corneum lipid hydrocarbon chains.[4,14] As seen in the spectra of (a) to (e), treatment with oleic acid in PG and oleylamine in PG resulted in significant shift toward higher

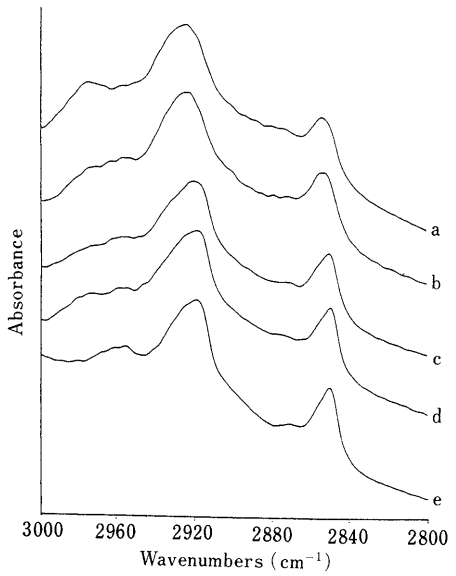

Fig. 1. Representative FT-IR/ATR Spectra of Rat Abdominal Stratum Corneum in the CH_2 C–H Asymmetric Stretching Region Following 2 h-Pretreatment with (a) 0.15 M Oleylamine in PG, (b) 0.15 M Oleic Acid in PG, (c) 1% Indomethacin in PG, (d) PG Alone, and (e) without Treatment

wavenumbers with the CH_2 C–H asymmetric stretching as compared with the untreated sample along with broadening of the spectra at 2919.8 ± 0.2 cm^{-1}. A similar shift was not observed with either PG or indomethacin in PG. Such frequency changes were possibly due to a disruption of lipid structures of the stratum corneum associated with incorporation of penetrants into the lipid domains.[5,15,16] The observed broadening of the spectra was considered to be due to a possible appearance of another peak arising from CH_2 C–H stretching associated with changes in the environment surrounding lipids treated with penetrants in PG. Oleic acid was reported to be incorporated into the human stratum corneum.[6] The CH_2 moieties of the incorporated oleic acid and PG into the stratum corneum domains in rat may be able to contribute to the shift toward the higher wavenumbers together with the CH_2 moieties of the native stratum corneum lipid hydrocarbon chains. However, there is no evidence to prove this hypothesis because of the difficulty in separating of stratum corneum lipid hydrocarbon chains from those of the incorporated PG and penetrants after pretreatment. As for the stratum corneum lipid composition, it was mentioned that the stratum corneum lipids are composed mainly of ceramides, cholesterol, fatty acids and cholesteryl esters, with ceramides amounting to more than 40% of all lipids in human and pig.[1] The number of methylene groups in a ceramide molecule is considerably greater than that in each molecule of the penetrant or PG. The ratio of ceramides to all of the lipids in rat stratum corneum may be similar to those in human and pig.[1] We can also hypothesize that the methylene moieties of penetrants and/or PG incorporated into the stratum corneum are probably small in quantity as compared with those of native stratum corneum lipids. Thereby, the CH_2 C–H asymmetric stretching vibrations arising from the penetrant-PG treated stratum corneum lipids may be primarily due to methylene groups of stratum corneum lipids, and the incorporated penetrant and/or PG

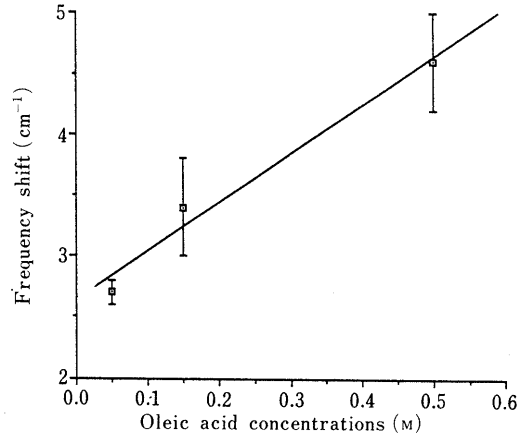

Fig. 2. Effects of Oleic Acid Concentrations on the Frequency Primarily Due to CH_2 C–H Asymmetric Stretching Vibrations Arising from the Rat Stratum Corneum Lipids

$r=0.975$.

TABLE I. Summary of Spectral, Flux, and Lag Time Changes Following Treatment of Rat Abdominal Skins with Fatty Acids and Fatty Amines Dissolved in PG

Treatment	IR frequency (cm^{-1})	Indomethacin flux ($\mu g/cm^2/h$)	Lag time (h)
Lauric acid	2922.2±0.6 (3)	30.3±2.5 (3)	3.3±1.3 (3)
Myristic acid	2921.8±0.4 (3)	11.2±3.0 (3)	5.7±0.3 (3)
Palmitic acid	2921.0±0.4 (3)	7.4±1.6 (3)	4.7±0.3 (3)
Stearic acid	2920.0±0.2 (3)	1.3±0.4 (3)	nd[a]
Oleic acid	2924.2±0.5 (3)	41.2±6.6 (6)	0.5±0.3 (6)
Laurylamine	2922.5±0.3 (3)	29.0±4.3 (3)	3.3±0.7 (3)
Myristylamine	2921.9±0.6 (5)	24.6±5.6 (4)	2.7±0.7 (4)
Hexadecylamine	2919.9±0.4 (3)	6.1±1.7 (4)	nd[a]
Stearylamine	2919.8±0.2 (3)	2.6±0.4 (3)	nd[a]
Oleylamine	2923.5±0.2 (3)	34.6±3.5 (5)	1.8±0.2 (5)
Azone	2922.2±0.4 (3)	27.4±4.3 (4)	5.2±1.0 (4)
PG alone	2919.8±0.2 (7)	1.4±0.6 (4)	7.9±0.2 (4)
No treatment	2919.8±0.2 (12)	—	

The values given are mean±S.D. Number of trials are given in parentheses. *a*) Not detected. Fatty acids and fatty amines were dissolved in PG and then used as the penetrant.

present in the stratum corneum may be a minor contributor. This interpretation is supported by our experimental results pursuing the concentration dependency of oleic acid on the frequency changes near 2920 cm^{-1} after 2 h treatment of the rat stratum corneum, where fairly good linearity between oleic acid concentration and the frequency shift was observed (Fig. 2). The degree of the shift in wavenumbers ranged approximately from 1 to 4.5 cm^{-1} depending upon the oleic acid concentrations, and at an oleic acid concentration of 0.5 M, the absorbance peak was 2924.4± 0.4 cm^{-1}. While, the FT-IR spectra of oleic acid (pure sample) shows the maximum peak at 2924.3±0.2 cm^{-1} resulting from the CH_2 C–H asymmetric stretching band. These two values in the maximum absorbance were very similar. However, at lower concentrations of oleic acid, the maximum absorbance resulting primarily from the CH_2 C–H asymmetric stretching vibrations of hydrocarbon chains of the stratum corneum lipids was lower than that of the pure oleic acid sample. Therefore, the peak in absorbance near 2920 cm^{-1} resulting from the stratum corneum samples treated with oleic acid could be explained as being due mainly to the constituents of the stratum corneum lipids, and it was rational to consider that the similarity in the above described two values was coincidental. With oleylamine, the maximum peak due to a CH_2 C–H asymmetric stretching vibration was 2922.4± 0.2 cm^{-1}, and this value was lower than the values obtained from the stratum corneum lipid samples treated with oleylamine (Table I).

One objective of our study was to pursue the relationship between the degree of stratum corneum disruption induced by penetrants and enhanced permeation of selected solutes. Indomethacin was chosen as the model lipophilic diffusate to examine the effects of penetrants on the stratum corneum lipid domains. Azone was introduced as one of the model penetrants, together with fatty acids and fatty amines. The CH_2 C–H asymmetric band position, indomethacin flux and lag time are summarized in Table I. When the skin was treated with 0.15 M oleic acid in PG, a shift in the asymmetric CH_2 C–H stretching band position from 2919.8±0.2 to 2924.2±0.5 cm^{-1} were observed. The CH_2 C–H stretching region of the FT-IR spectra of the skin samples treated with 1% indomethacin was not different in either shape or the wavenumber position (Fig. 1). Thus, it was assumed that 1% indomethacin in PG solution did not result in any perturbation of the stratum corneum lipid domains. The addition of oleic acid into the PG vehicle enhanced indomethacin flux from 1.4 (untreated sample) to 41.2 $\mu g/cm^2/h$. The lag time also decreased from 7.9 to 0.5 h. These results suggested that the structural perturbation caused by oleic acid in the stratum corneum domain might be related to indomethacin flux through the skin. Similar shifts of CH_2 C–H stretching band positions were noted for other fatty acids and fatty amines except for stearic acid, hexadecylamine and stearylamine. Oleic acid with an unsaturated –C=C– double bond in the alkyl chain enhanced the indomethacin flux and shortened the lag time significantly as compared to stearic acid, which has a saturated alkyl chain ($p<0.01$). Azone induced the disordering of the stratum corneum lipid domains as previously reported by several investigators.[17,18] Our results (Table I) also suggested a higher wavenumber shift in the PG vehicle with the presence of Azone. Azone increased indomethacin flux and decreased lag time. The increased flux and decreased lag time obtained in the presence of Azone was similar to that obtained for laurylamine, but Azone was less effective than oleic acid.

To determine whether there was a possible relationship between the spectral alteration in the CH_2 C–H stretching region, and the flux and lag time for indomethacin permeation, the wavenumber positions of asymmetric CH_2 C–H stretching vibrations were plotted as a function of indomethacin flux and lag time in Figs. 3A and 3B, respectively. The straight line in each plot represents the best linear fit of the data with correlation coefficients of 0.955 and of 0.902 for the flux and lag time plots, respectively. These correlations suggested that the degree of steric disordering within stratum corneum lipid domains was related to the transdermal indomethacin flux and the lag times; thus, spectral alteration in the CH_2 C–H stretching regions of rat full thickness skin may provide a reliable index for characterizing penetrants.

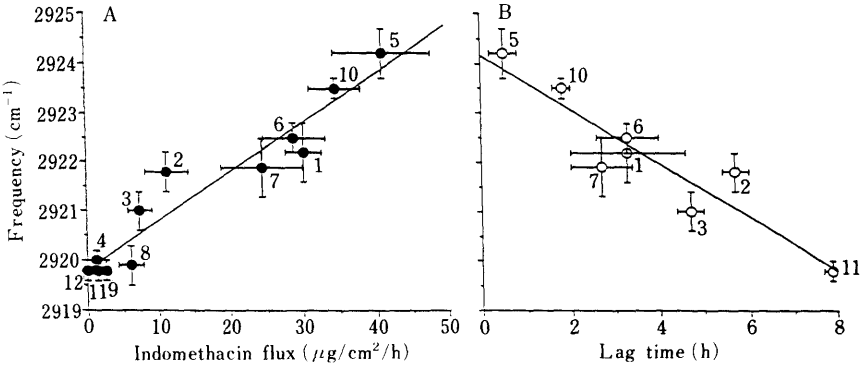

Fig. 3. Relationship between the Wavenumber Positions of CH_2C–H Asymmetric Stretching Peak of the Stratum Corneum and the Transdermal Flux of Indomethacin through the Rat Abdominal Skin, and the Lag Time for the Penetration through the Skin

The values listed in Table I were plotted to obtain these two figures. A, data for transdernal flux; B, data for the lag time. 1, lauric acid; 2, myristic acid; 3, palmitic acid; 4, stearic acid; 5, oleic acid; 6, laurylamine; 7, myristylamine; 8, hexadecylamine; 9, stearylamine; 10, oleylamine; 11, PG alone; 12, no treatment. A: $r=0.923$. B: $r=0.864$.

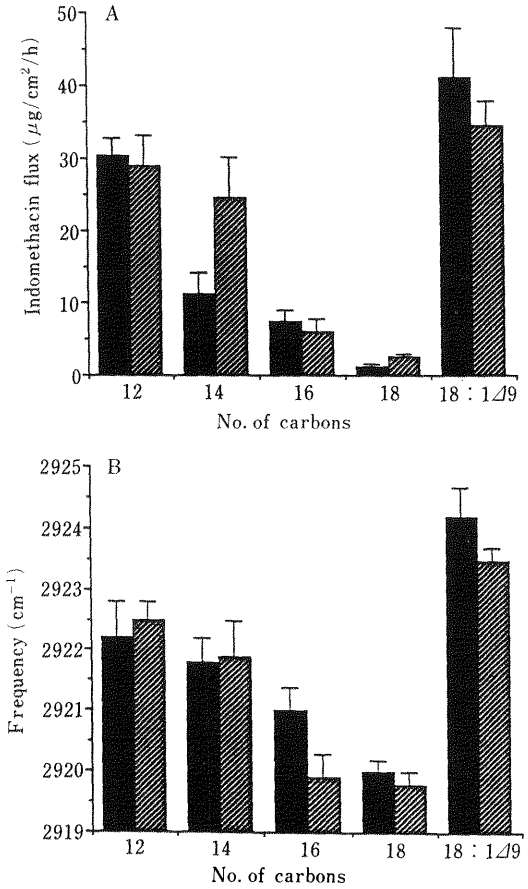

Fig. 4. Comparison of the Structural Difference of Fatty Acids and Fatty Amines for the Increased Indomethacin Flux through Rat Full Thickness Skin and Increased Frequency Primarily Due to CH_2 C–H Asymmetric Stretching of the Stratum Corneum Lipids

A, data for indomethacin flux: ■, for fatty acids; ▨, for fatty amines. B, data for increased frequency of CH_2C–H asymmetric stretching of the stratum corneum lipids: ■, for fatty acids; ▨, for fatty amines.

As shown in Figs. 4A and 4B, within both saturated fatty acid and saturated fatty amine analogues, decreasing the alkyl chain length from C_{18} to C_{12} resulted in a gradual and significant enhancement of the steady-state indomethacin flux and a bathochromic shift of the CH_2 C–H stretching band at 2920 cm^{-1}; the C_{12} analogs showing the maximum effect. Although saturated C_{18} analogs, stearic acid and stearylamine were not effective, corresponding monosaturated analogs, oleic acid and oleylamine demonstrated a very pronounced effect, even surpassing the effect of C_{12} compounds, lauric acid and laurylamine. It was also noted that there was no significant difference in the steady state indomethacin flux between fatty acids and fatty amines with the same carbon number in their structures. Aungst *et al.* reported that the magnitude of enhancement in skin penetration of naloxone was related to the chain length of the hydrophobic groups of both fatty acids and alcohols, which were used as penetrants, and they also hypothesized that fatty acid chains shorter than those having 16 or more carbon atom hydrophobic groups, which are the main components of fatty acids in the stratum corneum taken up into the skin, disrupt the crystalline lipid packing, resulting in a more fluid and permeable membrane.[19] Our above described linear relationship was in good agreement with their data on fatty acids and further indicated that the amino group of fatty amines and the carboxyl groups of fatty acids were not determinant factors for the alteration of stratum corneum structures. Based on our results where either unsaturated oleic acid or oleylamine treated skin showed a remarkable increase in indomethacin permeability, it may be speculated that these two penetrants, which are almost fully disordered in the stratum corneum,[20] exert their influences on the hydrophobic region of the lipid bilayers, resulting in the enhancement of over all steric disordering of stratum corneum lipids. A similar mechanism can be applied to other fatty acids and fatty amines in their skin permeability enhancing action. However, the question of the mechanism of such enhancement remains as a topic of debate. Apart from the mechanism of the skin peameability enhancing action of penetrants, our results stressed that the spectral alteration in CH_2 C–H asymmetric stretching regions of stratum corneum lipids may provide a reliable index for characterizing penetrants.

Figure 5A illustrates the FT-IR/ATR spectrum of the amide I region of stratum corneum treated with (a) 0.15 M oleylamine in PG, (b) 0.15 M oleic acid in PG, (c) PG alone for 2 h, respectively and (d) untreated skin sample, respectively. The amide I vibrational modes absorbing near 1640 cm^{-1} are sensitive to the conformation of proteins.[21–24] A shoulder near 1630 cm^{-1} appeared following a 2-h oleylamine and oleic acid treatment (Fig. 5A). When the skin was soaked for 2 h in PG alone in the process of preparing an IR sample of the excised skin, a protein

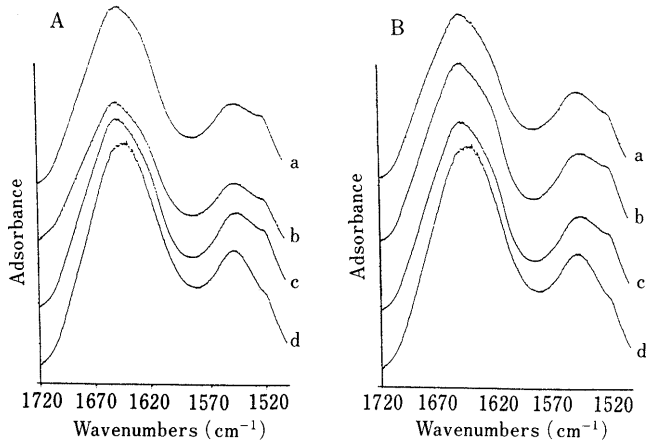

Fig. 5. Representative FT-IR Spectra of Rat Abdominal Stratum Corneum in the Amide I Region near 1640 cm^{-1} Originated from the Proteins Following 2 h-Treatment with Fatty Amines and Fatty Acids

A, the skin was treated with either: a, 0.15 M oleylamine in PG; b, 0.15 M oleic acid in PG; c, PG alone; d, without treatment. B, The skin was treated with either: a, 0.15 M laurylamine; b, 0.15 M lauric acid; c, PG alone; d, without treatment.

TABLE II. Effect of PG on the *in Vitro* Accumulation of CF in Rat Skin after 2 h-Pretreatment of PG

Vehicle	Accumulation of CF (ng/cm^2)
PBS	69.9± 8.7
PG	383.4±72.5[a]

Rat skin was pretreated with either phosphate buffer, pH 7.4 (PB) or PG for 2 h, and then 0.2 w/w% CF dissolved in PB (pH 7.4) was applied to the skin. The details are described in the experimental section. Each value represents the mean±S.D. of 3 experiments. *a*) Significantly different from PBS at $p<0.005$.

conformational change was also detected, but this change was smaller than the change observed in the samples treated with oleylamine and oleic acid. Similar but less pronounced results were also obtained from the skin samples treated with lauric acid and laurylamine, respectively (Fig. 5B). Similar conformational changes in the amide I region within human stratum corneum have already been reported with alkyl sulfoxide.[25] Based on this finding, it is suggested that both oleylamine and oleic acid–PG systems and laurylamine and lauric acid–PG systems are also able to alter the conformation of stratum corneum keratinized proteins. It is probable that such conformational changes of stratum corneum keratinized proteins are possibly the result of an incorporation of oleic acid, oleylamine and PG, which may also induce the reorganization of stratum corneum lipid structures. As with PG, it was reported that PG probably works by solvating alpha-keratin and occupying hydrogen-bonding sites, thus reducing drug/tissue binding.[18] The result of our FT-IR/ATR analysis indicated that PG altered the stratum corneum protein structures from alpha- to beta-forms, though the degree of the structural alteration was not clear, would substantially support the explanation that PG act on stratum corneum proteins.

An *in vitro* accumulation of hydrophilic compound, CF, in the presence of PG in rat skin was studied (Table II). The *in vitro* accumulation of CF within the PG pretreated samples is significantly greater than within the untreated samples, thus indicating that PG possibly enhances the accumulation of the polar solute, CF. The enhanced skin accumulation of hydrophilic compounds such as CF is presumably associated with an alteration of the conformation of stratum corneum proteins. Yamada *et al.* have suggested that permeation of molsidomine was increased as a result of oleic acid and PG penetration into the stratum corneum, improving the permeability of the skin by dissolving hard lipoidal components, and thus facilitating the transport of drug dissolved in PG through the modified stratum corneum.[26] Our results suggest the possible contribution of skin protein being perturbed by PG in the accumulation of CF, a polar solute.

In summary, the treatment of skin with either oleic acid of oleylamine resulted in a significant CH_2 C–H asymmetric stretching band shift and broadening. The extent of spectral alteration varied with the chemical structure of the penetrant (fatty acids and fatty amines). The plot of frequency changes *vs.* indomethacin flux or the lag time demonstrated a linear relationship. From this data, we may concluded that the frequency changes could be used as an index to evaluate the ability of penetrants. We may also concluded that the chain length of the hydrophobic group in fatty acids and fatty amines could be a determinant factor for enhancing the perturbation of the stratum corneum structure. The hydrophilic amino and carboxyl groups attached to the CH_2 group in the penetrant molecules did not play a part in membrane permeability enhancing action. Oleic acid, oleylamine and PG appeared to induce a conformational alteration of keratinized proteins from α-helix to beta sheet. Accumulation of CF was significantly increased by the PG pretreatment of the skin, thus suggesting that PG-induced protein conformational changes could be related to the enhancement of CF accumulation.

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